REMARKS

Licensing and Review issued a form PTOL-456 on June 12, 2002 for the above-identified application, taking the position that the disclosed invention may be "useful in the production or utilization of special nuclear material or atomic energy", citing 42 U.S.C. 2182. Applicants respectfully disagree and respectfully overcome the basis for this communication in view of the discussion presented *infra.*

First, as noted in the FIELD OF THE INVENTION, the above identified invention discloses the following subject matter:

> The present invention relates in part to isolated nucleic acid molecules (polynucleotide) which encodes a human nuclear receptor proteins, referred to throughout as nNR7 and nNR7-1, respectively. The present invention also relates to recombinant vectors and recombinant hosts which contain a DNA fragment encoding nNR7 and nNR7-1, substantially purified forms of associated human nNR7 protein and associated human nNR7-1 protein, human mutant proteins of nNR7 and nNR7-1, and methods associated with identifying compounds which modulate nNR7 and nNR7-1 activity.

The undersigned attorney presumes that this notice was issued in light of the use of the term "nuclear" throughout the application. As sampled above, the term "nuclear receptor" is used throughout the present application to denote a class of biological proteins which have been shown to effect gene expression by acting as transcription factors. This concepts is introduced in the first paragraph of the BACKGROUND OF THE INVENTION, as follows:

> *The nuclear receptor superfamily, which includes steroid hormone receptors, are small chemical ligand-inducible transcription factors which have been shown to play roles in controlling development, differentiation and physiological function.* Isolation of cDNA clones encoding nuclear receptors reveal several characteristics. First, the NH2-terminal regions, which vary in length between receptors, is hypervariable with low homology between family members. There are three internal regions of conservation, referred to as domain I, II and III. Region I is a cysteine-rich region which is referred to as the DNA binding domain (DBD). Regions II and III are within the COOH-terminal region of the protein and is also referred to as the ligand binding domain (LBD). For a review, see Power et al. (1992, *Trends in Pharmaceutical Sciences* 13: 318-323).[1]

[1] Emphasis added. Also, Power, et al. is attached (Exhibit A) as a review of nuclear receptors.

Therefore, the term "nuclear" as used throughout this application denotes a component of a biological system, not an atomic one.

Applicants also respectfully note that a Foreign Filing License was granted in the parent application, U.S. Application Serial No. 09/209,069, filed December 10, 1998. A Foreign Filing License was granted in the '609 application on January 28, 1999. A copy of the corrected Filing Receipt in that application is attached as Exhibit B.

Therefore, Applicants respectfully take the position that this application does not relate to any "special nuclear material or atomic energy", and thus, no Formal Requirement should be issued in regard to the above-identified application. The Licensing and Review group is encouraged to contact the undersigned attorney with any questions or comments.

Respectfully submitted,

J. Mark Hand

J. Mark Hand
Reg. No. 36,545
Attorney for Applicants
Merck & Co., Inc.
P.O. Box 2000
Rahway, NJ 07065-0907
(732) 594-3905

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New insights into activation of the steroid hormone receptor superfamily

Ronan F. Power, Orla M. Conneely and Bert W. O'Malley

For many years the prevailing view of how steroid hormone receptors exert their effects on gene transcription has been that these intracellular receptor proteins, upon association with their specific cognate ligands, undergo a transformation to a state where they are capable of interacting with chromatin and regulating the transcription of specific genes. It has become implicit dogma in the field of biochemical endocrinology that receptor activation is absolutely dependent upon this ligand-binding event. However, recent studies described here by Bert O'Malley and colleagues *have shown that certain members of the steroid hormone receptor superfamily can be activated in a totally ligand-independent manner by a cell membrane receptor agonist, the neurotransmitter dopamine.*

Steroid hormone receptors are members of a superfamily of ligand-inducible transcription factors that also includes the receptors for thyroid hormone, retinoic acid and vitamin D_3 (Ref. 1). The classical model of steroid hormone action has been known for over 15 years and involves the entry of hormone into a cell by passive diffusion, where it binds to and induces a conformational change in its cognate receptor protein. This leads to intranuclear translocation, interaction with chromatin and modulation of gene expression[2–4]. It has long been accepted that these specific intracellular receptors are activated only upon binding their cognate ligands.

Over the past five to six years the cDNAs for all of the major receptor proteins within this superfamily have been cloned and sequenced[1]. The N-terminal region of the receptors is hypervariable and displays low homology between the receptor proteins. This domain varies in length from 25 amino acids for the vitamin D receptor to 603 amino acids for the mineralocorticoid receptor. The epitopes of antibodies raised against receptors are usually located within this hypervariable region.

R. F. Power is Research Associate, O. M. Conneely is Assistant Professor and B. W.

A further comparison of the deduced amino acid sequences for these receptors reveals three major internal regions (I–III) of amino acid conservation common to all members of this family[5]. Region I is a highly conserved 66 amino acid sequence that constitutes the DNA-binding domain. This region contains nine cysteine residues, eight of which are believed to form two zinc fingers with each finger containing one zinc atom. The DNA-binding domain of the receptor contains the sequences that recognize the specific steroid response elements that are located in the flanking regions of target genes. It is believed that the more N-terminal zinc finger determines the target gene or steroid response element sequence specificity while the more C-terminal zinc finger contacts the sugar–phosphate backbone of the DNA helix to stabilize binding of receptor to the steroid response element[4]. It is this subclass (type II) of zinc finger motif that distinguishes the steroid receptor superfamily from other transcription factors, such as the amphibian TFIIIA that belongs to a class of DNA-binding transcription factors using two cysteine and two histidine residues to bind coordinately one zinc atom[6].

The other two major regions of conserved amino acids (II and III) are located within the C-terminal or ligand-binding domain of the amino acids, respectively, and are hydrophobic. They are obviously of functional importance since they are conserved to some degree among all members of the superfamily. It remains unclear whether they are involved directly in ligand binding, dimerization, or transcriptional activation via protein–protein interactions. Dissection of the C-terminus or ligand-binding domain by mutational analysis has proved difficult.

Functional map of steroid receptors

Taken together, a large number of mutational analyses on separate receptors provides us with a general appreciation of structure–function relationships of domains of members of the steroid superfamily. The major functional domains of steroid/thyroid/vitamin receptors are presented schematically in Fig. 1. Although the basic structural regions of intracellular receptors are often subdivided to a greater degree (as described later), there is probably agreement on four distinct structural regions that give rise to eight or more separable functions. The more centrally located DNA-binding domain represents the structural hallmark for members of this family and is one of three highly conserved amino acid stretches among all members of the superfamily[6].

Ligand binding is relegated to the C-terminal domain (Fig. 1, domain 4) and probably involves multiple amino acid contacts with ligands over a broad region[5]. Agonists and antagonists do not have identical contact amino acids; critical contacts for antagonists appear to be located more toward the N-terminal within domain 4 and agonists more toward the C-terminal tail. DNA binding is provided almost wholly via amino acid contacts of domain 2 with specific DNA sequences. Following DNA binding, at least two domains contribute to transactivation of target genes by protein–protein interactions with the transcriptional machinery. These regions are variable between members of the family and the precise amino acid contacts are not obvious for any given member. It does appear, however, that

The region of domain 1 nearest the N-terminal often contains a specific sequence that appears to direct transactivation to certain genes; the mechanism of this preference is unknown but is probably via protein–protein interactions with transfactors at target genes[4]. Recent evidence in our laboratory has implicated the extreme C-terminal tail as a repressor of transactivation. This structural repressive function is overcome by hormonal agonists but not by antagonists, an observation that may be intrinsic to their distinct activities.

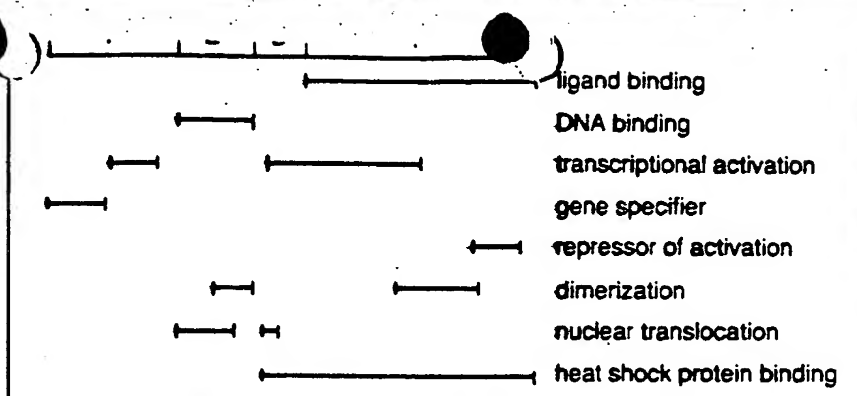

Fig. 1. Structural and functional domains of members of the steroid receptor superfamily.

At least two dimerization domains have been described for members of this superfamily (Fig. 1). One is located in the C-terminal region of domain 2 and another more toward the terminus of domain 4. Dimerization is critical for tight binding to the steroid response element of target genes; heterodimers between members of this superfamily, especially the smaller intracellular receptors, are not uncommon and appear to provide another level of functional diversity. Nuclear translocation domains are located in domains 2 and 3 and aid in translocation and retention of these molecules in the nucleus where they must act. Finally, a broad expanse of the C-terminal domain has been implicated in the binding of a variety of heat shock proteins (hsp90, hsp70, hsp56, etc.). These proteins are thought to be involved in promoting proper folding of nascent receptors and perhaps in chaperoning them to their intracellular locations.

Orphan receptors

The close structural relatedness that runs throughout the steroid receptor superfamily prompted a search for new members of this class of transcription factors. This was initiated by cross-screening, at low hybridization stringency, cDNA libraries using sequences conserved among the receptor family and in particular sequences from the highly conserved DNA-binding domain. The application of this technology has led to perhaps the most exciting development in this field in recent years, namely the discovery of a large number of receptor-related proteins comprising the steroid receptor superfamily. These newly discovered receptoroids satisfy all criteria of amino acid sequence conservation for classification as authentic members of the steroid receptor class of transcription factors.

The discovery of these new receptoroids raised two main questions. First, what function do they perform? Secondly, if they are ligand-activated transcription factors like the classical steroid receptor family members to which they are related, what is the nature of these ligands? These mostly unanswered questions have led to the terminology 'orphan receptors' to describe these new proteins with unknown ligands or functions. The first published orphan receptors were termed estrogen receptor related (ERR) 1 and 2 (Ref. 7) and were isolated using the DNA-binding domain of the estrogen receptor as a hybridization probe. Although discovered over three years ago, these two receptors are still of unknown function.

Since this first report, there has been a large increase in the number of orphan receptors identified in species as diverse as *Drosophila* and man. For example, the transcription factor called chicken ovalbumin upstream promoter transcription factor (COUP-TF) was found to be an authentic member of the steroid receptor superfamily[8]. This transcription factor participates in the regulation of several genes including the chicken ovalbumin gene, the rat insulin II gene, the pro-opiomelanocortin gene and the apo-very low density lipoprotein II gene[9–12].

When COUP-TF was cloned, it was noted that its sequence contained a twin zinc finger DNA-binding domain with the amino acid sequence in this region showing homology in 20 of 20 diagnostic amino acids and 11 of 12 conserved amino acids derived as a consensus for the steroid receptor superfamily. Additional significant homologies to steroid receptor family members were also observed in regions II and III of the COUP-TF protein. A schematic representation of selected published sequences for orphan receptors set against COUP-TF as a reference point is shown in Fig. 2. Any such list, however, is bound to be incomplete due to the continuing rapid expansion of this receptor subgroup and the fact that many orphan sequences remain unpublished or are in the process of publication.

The fact that a number of functions had already been ascribed to COUP-TF prompted an attempt to declassify further this receptor from the orphan subgroup by initiating a search for a ligand or activator for this transcription factor. Although no direct evidence for ligand-dependent activation of COUP-TF existed, the level of sequence conservation between the C-terminus of COUP-TF and the C-termini of more classical steroid receptors suggested that this was a distinct possibility. Furthermore, the *Drosophila* analog of human COUP-TF, termed seven-up (Ref. 13, Fig. 2), shares a 93% amino acid identity with the human protein

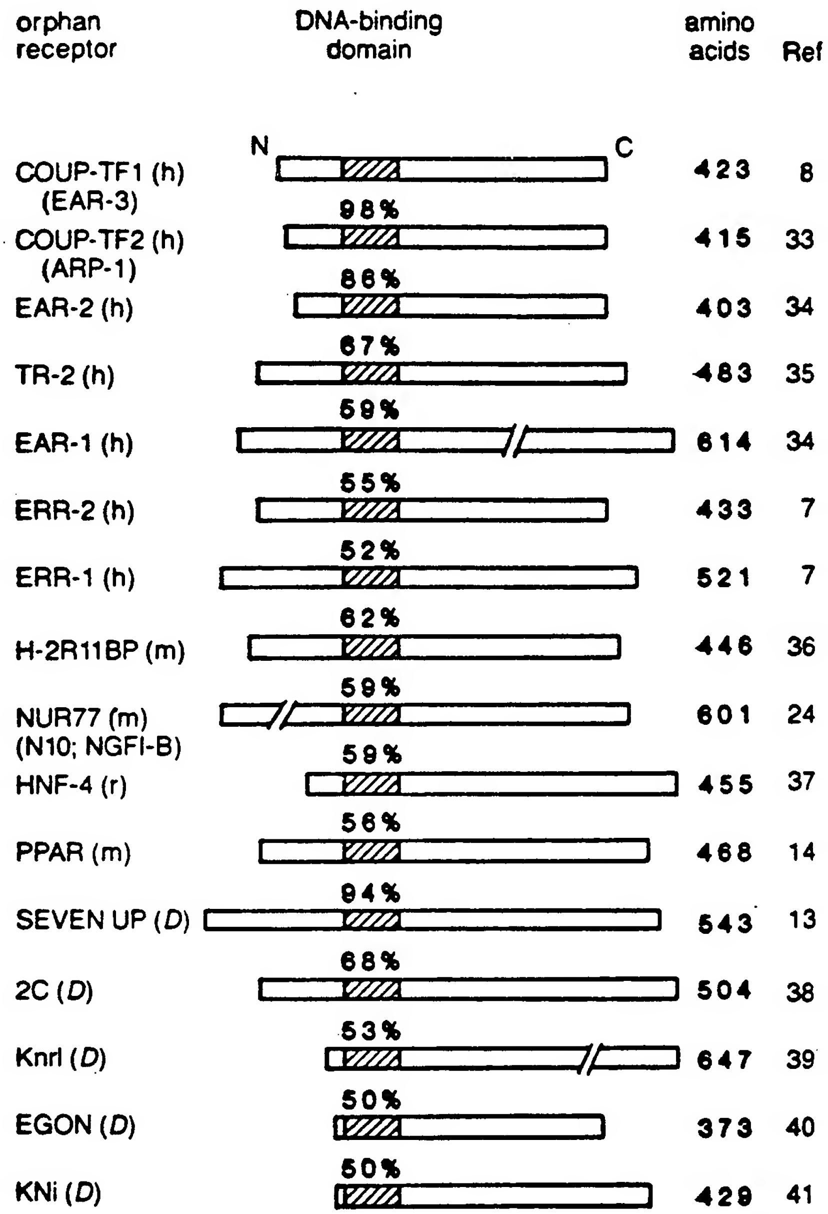

Fig. 2. Sequence homologies of orphan receptors. The **DNA-binding domains** *of the receptors are aligned and per cent sequence homologies are given relative to the human transcription factor COUP-TF (see text). These orphan receptors were isolated from species as diverse as* Drosophila (*D*), *rat* (r), *mouse* (m) *and human* (h).

in its C-terminal region. This level of interspecies sequence conservation suggested a conservation of function and perhaps ligand-dependent activation. Finally, a recent study has shown that one of the members of this orphan receptor subgroup can be activated by exogenously added compounds[14]. This orphan receptor from mouse, termed the peroxisome proliferator activated receptor (PPAR) was activated in transient transfection assays by peroxisome proliferators, which are a diverse class of hepato-

study showed that orphan receptors had the potential to be activatable transcription factors.

To assess the ability of potential ligands to regulate COUP-TF, a chimera was made in which only the DNA-binding domain (i.e. 2) of COUP-TF (see Fig. 1) was swapped for that of progesterone, using recombinant techniques. This allows activation of a target gene that contains the progesterone response element without interference with endogenous COUP-TF in transfected cells.

transient transfection assay in a monkey kidney cell line, CV1 (Ref. 15). Compounds included adrenal and gonadal steroids and their major metabolites. All fat-soluble and water-soluble vitamins were tested, as were retinoic acid and derived compounds. Surprisingly, of these compounds, the neurotransmitter dopamine stimulated COUP-TF-dependent activation of an appropriate target gene in the assay system. Competitive binding assays showed that dopamine was not binding directly to COUP-TF and suggested that activation of the COUP-TF chimeras by this neurotransmitter was a result of an indirect signalling pathway.

Dopamine action is mediated by its interaction with membrane-bound receptors coupled to G proteins. Several dopamine receptors have been identified that belong to two main receptor subtypes, D_1 and D_2, that stimulate and inhibit adenylyl cyclase, respectively[16]. The presence of dopamine receptors of the D_1 subtype in renal tissue that are coupled to either adenylyl cyclase or the phospholipase C signalling pathway has been documented[17], and it has been demonstrated that the CV1 monkey kidney cells used in transfection assays possess a dopamine-sensitive adenylyl cyclase activity[18]. It is therefore possible that dopamine activation of COUP-TF is mediated by phosphorylation. Consistent with this hypothesis was the finding that 8-bromo-cAMP and the potent inhibitor of protein phosphatases 1 and 2A, okadaic acid, effectively mimicked the action of dopamine by eliciting COUP-TF-dependent activation of a target gene.

The finding that a catecholamine neurotransmitter was capable of activating an authentic member of the steroid receptor superfamily appears to be the first direct evidence of 'cross-talk' between these two very different signal transduction pathways, and demonstrated that a neurotransmitter could have potential access to the genome via a highly evolved and potent family of transcription factors. Interestingly, preliminary studies (O'Malley, B. W. *et al.*, unpublished) show that COUP-TF is

Ligand-independent activation

The concept of a membrane receptor agonist activating an intracellular orphan member of the steroid receptor family prompted an investigation of whether more classical steroid receptors could be activated via membrane-associated signalling pathways. Indeed, a retrospective survey of the literature over the past few years raises several questions about the absoluteness of the classic steroid hormone–steroid receptor paradigm. In particular, it has been known for several years that intravenous or intracerebral administration of progesterone can facilitate female mating behavior (lordosis) in estrogen-primed rats[19]. It was demonstrated that dibutyryl cAMP can substitute for progesterone in eliciting such behavior[20]. More recently it has been shown that the oncogenic derivative of the thyroid hormone receptor (erb-A), which does not bind thyroid hormone, requires phosphorylation at certain sites in order to function as an oncogene[21]. In addition, 8-bromo-cAMP and okadaic acid can mediate avian progesterone receptor-dependent transcription in the absence of progesterone[22]. Finally, many laboratories have shown that classical steroid receptors are phosphoproteins, and recent results indicate that the final common denominator in ligand-mediated receptor activation is a nuclear, DNA-dependent phosphorylation of receptor[23]. These observations indicate that certain steroid receptor effects may be mediated not via steroidal ligands but via modified intracellular second messenger pathways.

Dopamine is capable of selectively activating other members of the steroid receptor superfamily in a totally ligand-independent manner in a transient transfection assay system[18]. This was true for both forms, A and B, of the chicken progesterone receptor, for the human estrogen receptor and the human vitamin D receptor. Initial studies also indicated that human thyroid hormone receptor β can mediate ligand-independent transcription from a relevant target demonstrated that dopamine could mimic the effect of progesterone in translocating this receptor from cytoplasm to nucleus (O'Malley, B. W. *et al.*, unpublished).

As stated above, a dopamine-sensitive adenylyl cyclase was present in the CV1 host cells used in these assays. Dopamine-dependent activation of the progesterone receptor, at least, appeared to occur through stimulation of D_1 subtype receptors, based on the observation that a selective D_1 receptor agonist, SK&F38393, stimulated progesterone receptor-dependent transcription in the assay system while a selective D_2 receptor agonist, quinpirole, did not. Furthermore, the results with the chicken progesterone receptor indicated that mutation of a specific serine residue in the C-terminus of the receptor effectively blocked the ability of dopamine to activate receptor-dependent transcription but had no effect on the progesterone-stimulated transcriptional response of this mutant relative to the wild-type receptor. This result indicates that a dual and dissociable activation mechanism exists for the progesterone receptor and probably for the other dopamine-responsive steroid hormone receptor family members.

Interestingly, not all steroid receptors were activated by this neurotransmitter under test conditions (O'Malley, B. W. *et al.*, unpublished). CV1 cells co-transfected with a human glucocorticoid expression vector and an appropriate target gene were transcriptionally responsive to dexamethasone but were totally unresponsive to dopamine. Likewise, cells transfected with a human mineralocorticoid expression construct were only marginally responsive to dopamine. The level of specificity of the response of, for example, chicken progesterone receptor to dopamine was further underlined by the finding that although CV1 cells possessed a β-adrenoceptor-responsive adenylyl cyclase in addition to a dopamine-sensitive adenylyl cyclase, no transcriptional response was observed with the β-receptor agonist isoprenaline. This suggests a more complex activation mechanism than just an elevation lation of the phospholipase C pathways. In this mechanism, generation of the second messengers inositol-1,4,5-trisphosphate and diacylglycerol could implicate protein kinase C or calmodulin-dependent protein kinase as the transducing factors that couple dopamine stimulation of cell surface receptors to the activation of steroid hormone receptors. Obviously the elucidation of the precise signalling pathway involved in dopamine's activation of these intracellular receptors will be an important target of future work.

New perspectives on steroid receptor superfamily

Based on these findings it may be necessary to re-evaluate our current understanding of the molecular pathway of steroid hormone action. Such studies do not invalidate the current concepts of ligand activation of steroid receptor family members but extend the concept of activation to membrane receptor-mediated phosphorylation pathways or cascades (Fig. 3). The duality of this mechanism may not be absolute. Thus steroid receptors such as those for glucocorticoids and mineralocorticoids may not possess this alternative activation capability and may be solely activated by their cognate ligands. On the other hand, some orphan members of this family may not be ligand activated but may be activatable only by a membrane signalling pathway as described above. COUP-TF and at least one other orphan receptor, NGFI-B (Ref. 24), may be activated via phosphorylation-mediated pathways. The gene encoding the latter protein is rapidly activated by a variety of stimuli, such as nerve growth factor or ion channel perturbations. It has recently been shown that this protein is rapidly phosphorylated post-translationally in response to growth-promoting compounds but remains unphosphorylated or underphosphorylated in unstimulated cells.

Some orphan receptors, such as COUP-TF, that are found in species as diverse as *Drosophila*, sea urchin and man, may represent the more primitive members of the steroid receptor superfamily. These types of receptors may have

arisen initially to modulate cell differentiation and development in primitive organisms, in response to simple stimuli such as an increase in intracellular cAMP concentration or stimulation of a phosphorylation-mediated pathway by binding of a simple, perhaps nutritionally derived, molecule at the cell surface. As time progressed these more primitive receptors may have evolved to become regulated by more specialized mechanisms, such as ligands produced in specific cells of plants, insects or vertebrates, while in some cases retaining their ancestral ability to be acted upon by other stimuli initiated at the plasma membrane. Consider, for example, that while retinoids are important morphogens in vertebrate development, cAMP is a major factor in inducing cell differentiation in the primitive slime mold *Dictyostelium*[25].

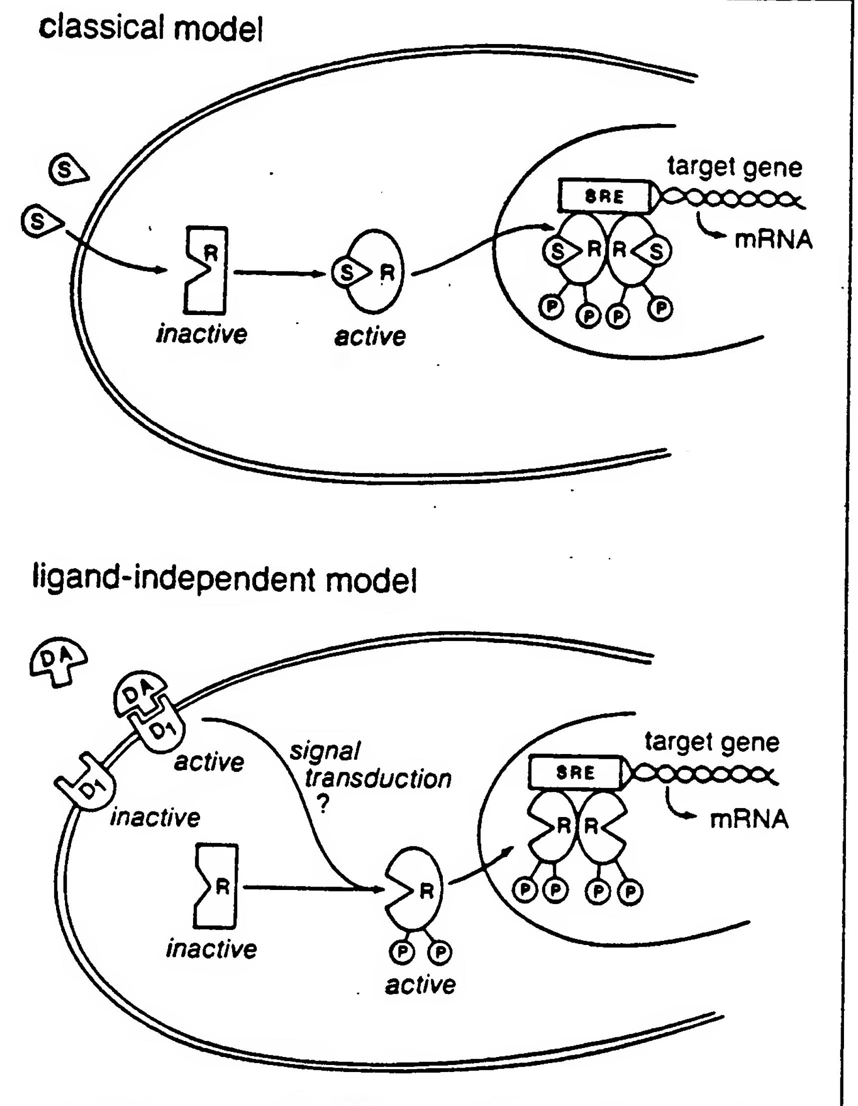

Fig. 3. Scheme of two models of steroid hormone action. In the **classical model**, *steroids* **(S)** *enter target cells by passive diffusion where they bind to and induce a conformational change in their cognate receptor proteins* **(R)**. *The active steroid–receptor complex translocates to the nucleus where it interacts with target genes by binding to steroid response elements* **(SREs)** *in the flanking regions of these genes. This leads to* **mRNA** *production, protein synthesis and altered cellular function. The* **ligand-independent model** *has a membrane receptor-associated activation mechanism. Binding of dopamine to* D_1 *dopamine receptors at the cell surface initiates an as yet unidentified signal transduction pathway that leads to activation of steroid receptors, presumably by phosphorylation-dependent mechanisms. The non-ligand-bound, active receptor translocates to the nucleus where it again modulates target gene transcription.*

Implications of ligand-independent activation

The demonstration of cross-talk between membrane-associated dopamine receptors and intracellular steroid receptors may have biomedical significance. It is probable that this ligand-independent activation of steroid receptor family members will be shown to occur via other membrane receptors or phosphorylation pathways. It also seems likely that examples of cell specificity and gene-selective activation will be found. Perhaps more likely, however, is the possibility that the classical intracellular receptor pathway would be enhanced (or repressed) by certain events that occur at the cell membrane and alter intracellular phosphorylation. Synergism is more likely than total activation from the membrane. Members of the steroid receptor family without known ligands, currently referred to as orphan receptors, appear to be special candidates for regulation by this or similar alternative mechanisms.

Dopamine in the CNS is associated with psychomotor and psychiatric disorders such as Parkinson's disease and schizophrenia[26], and it may also play a role in alcoholism and cocaine addiction[27,28]. In view of this, the may be important from a neuroendocrinological perspective. Dual activation of receptors by both intracellular and membrane-reactive ligands must be considered to be possible *in situ* in brain cells. This may help to answer such long-standing questions as the function of the specific androgen receptors that are known to exist in the cerebral cortex of female brain[29] or the progesterone and estrogen receptors that exist in regions of the male brain[30]. From family members that are known to exist in disease states, for example, in cases of hypocalcemic vitamin D-resistant rickets where decreased or absent ligand-binding is manifested[31].

At the level of a neurobiological understanding of learning and memory processes, the finding that a neurotransmitter has access to the genome via a highly evolved and potent superfamily of transcription factors could be of particular importance. A prevalent

systems. Predominant theori[illegible] based on studies using RNA [illegible] protein synthesis inhibitors, suggest that this process requires alterations in gene expression and protein synthesis that lead to changes in chronic neuronal or synaptic functions[32]. The next step will be to elucidate the biological effects of steroid receptors on gene expression in neuronal systems and to identify target genes activated by steroid receptors possessing membrane-associated activation mechanisms. Such a signalling pathway between membrane and steroid receptor family members provides new perspectives for our concepts of cell and molecular regulation and for the fields of molecular endocrinology, neurobiology and pharmacology.

References

1 Evans, R. M. (1988) *Science* 240, 889–895
2 Gorski, J., Toft, D., Shyamala, G., Smith, D. and Notides, A. (1968) *Recent Prog. Horm. Res.* 24, 45–80
3 O'Malley, B. W., McGuire, W. L., Kohler, P. O. and Korenman, S. (1969) *Recent Prog. Horm. Res.* 25, 105
4 Beato, M. (1989) *Cell* 56, 335–344
5 Carson-Jurica, M. A., Schrader, W. T. and O'Malley, B. W. (1990) *Endocr. Rev.* 11, 201–220
6 O'Malley, B. W. (1990) *Mol. Endocrinol.* 4, 363–369
7 Giguere, V., Yang, N., Segui, P. and Evans, R. M. (1988) *Nature* 331, 91–94
8 Wang, L-H. *et al.* (1989) *Nature* 340, 163–166
9 Wang, L-H., Tsai, S. Y., Sagami, I., Tsai, M-J. and O'Malley, B. W. (1987) *J. Biol. Chem.* 262, 16080–16085
10 Hwung, Y-P., Crowe, D. T., Wang, L-H., Tsai, S. Y. and Tsai, M-J. (1988) *Mol. Cell. Biol.* 8, 2070–2074
11 Drouin, J. *et al.* (1989) *Genome* 31, 510–519
12 Wijnholds, J. and Philipsen, J. N. (1988) *EMBO J.* 7, 2757–2760
13 Mlodzik, M., Hiromi, Y., Weber, U., Goodman, C. S. and Rubin, G. M. (1990) *Cell* 60, 211–224
14 Issemann, I. and Green, S. (1990) *Nature* 347, 645–649
15 Power, R. F., Lydon, J. P., Conneely, O. M. and O'Malley, B. W. (1991) *Science* 252, 1546–1548
16 Kebabian, J. W. and Calne, D. B. (1979) *Nature* 277, 93–96
17 Felder, C. C., Blecher, M. and Jose, P. A. (1989) *J. Biol. Chem.* 264, 8739–8745
18 Power, R. F., Mani, S. K., Codina, J., Conneely, O. M. and O'Malley, B. W. (1991) *Science* 254, 1636–1639
19 Kubli-Garfias, C. and Whalen, R. E. (1977) *Horm. Behav.* 9, 380–386
20 Beyer, C., Canchola, E. and Larsson, K. (1981) *Physiol. Behav.* 26, 249–251
21 Glineur, C., Zenke, M., Beug, H. and Ghysdael, J. (1990) *Genes Dev.* 4, 1663–1676
B. W. (1990) *Science* 250, 1740–1743
23 Weigel, N. L., Carter, T. H., Schrader, W. T. and O'Malley, B. W. *Mol. Endocrinol.* (in press)
24 Fahrner, T. J., Carroll, S. L. and Milbrandt, J. (1990) *Mol. Cell. Biol.* 10, 6454–6459
25 Kay, R. R. *et al.* (1988) *Dev. Genet.* 9, 579–587
26 Seeman, P. (1987) *Synapse* 1, 133–152
27 Blum, K. *et al.* (1990) *J. Am. Med. Assoc.* 263, 2055–2060
28 Dackis, C. A., Gold, M. S., Davies, R. K. and Sweeney, D. R. (1985) *Int. J. Psych. Med.* 15, 125–135
29 Sholl, S. A. and Pomerantz, S. M. (1986) *Endocrinology* 119, 1625–1631
30 Blaustein, J. D., Ryer, H. I. and Feder, H. H. (1980) *Neuroendocrinology* 31, 403–409
31 Hughes, M. R. *et al.* (1988) *Science* 242, 1702–1705
32 Matthies, H. (1989) *Prog. Neurobiol.* 32,
(1990) *Science* 251, 561–565
Miyajima, N. *et al.* (1989) *Cell* 57, 31–39
Zhang, C. and Kokontis, J. (1988) *Biochem. Biophys. Res. Commun.* 155, 971–977
36 Hamada, K. *et al.* (1989) *Proc. Natl Acad. Sci. USA* 86, 8289–8293
37 Sladek, F. M., Zhong, W., Lai, E. and Darnell, J. E. (1990) *Genes Dev.* 4, 2353–2365
38 Henrich, V. C., Sliter, T. J., Lubahn, D. B., MacIntyre, A. and Gilbert, L. I. (1990) *Nucleic Acids Res.* 18, 4143–4146
39 Oro, A. E. *et al.* (1988) *Nature* 336, 493–496
40 Rothe, M., Nauber, U. and Jackle, H. (1989) *EMBO J.* 8, 3087–3094
41 Nauber, U. *et al.* (1988) *Nature* 336, 489–491

SK&F38393: 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine

Designing therapeutically effective 5-lipoxygenase inhibitors

R. M. McMillan and E. R. H. Walker

Metabolism of arachidonic acid by the enzyme 5-lipoxygenase leads to the formation of a group of biologically active lipids known as leukotrienes. Peptidoleukotrienes are powerful bronchoconstrictor agents while leukotriene B_4 is a potent chemotactic agent for a variety of leukocytes. In view of these properties, leukotrienes have been proposed as important mediators in allergic and inflammatory disorders, and inhibitors of 5-lipoxygenase, by blocking leukotriene synthesis, have therapeutic potential in a range of diseases including arthritis and asthma. This review by Rodger McMillan *and* Ed Walker *summarizes the biology of leukotrienes and the current knowledge of the mechanism of 5-lipoxygenase, providing a framework for consideration of the discovery, development and clinical status of drugs in the three major classes of 5-lipoxygenase inhibitors: 'redox' inhibitors, iron ligand inhibitors and 'non-redox' inhibitors.*

Lipoxygenase pathways are widely distributed in the plant and animal kingdoms and are responsible for catalysing the oxidative metabolism of a range of unsaturated fatty acids. In mammalian systems, the predominant substrate is arachidonic acid, and lipoxygenases are classified according to the position at which they oxidize arachidonic acid. The mammalian 5-lipoxygenase pathway (Fig. 1) produces the potent biological mediators leukotriene B_4 (LTB_4) and the peptidoleukotrienes (LTC_4, LTD_4 or LTE_4), and this has stimulated the search for 5-lipoxygenase inhibitors.

5-Lipoxygenase has been purified from several sources and in each case activity is dependent on Ca^{2+} and ATP, a feature that distinguishes the enzyme from other lipoxygenases. The amino acid sequences of the human and rat enzymes have been derived from their respective cDNA clones. They are proteins of 674 amino acids with molecular masses of 78 kDa and there is 93% identity

R. M. McMillan is Section Manager, Bioscience Department 1, and E. R. H. Walker is Section Manager, Chemistry Department 1, Research Department 1, ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire, UK SK10 4TG.

EXHIBIT B - 10/090,090 - f[illegible] March 4, 2002

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J MARK HAND
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KATHLEEN KLUTKOWSKI

Receipt is acknowledged of this nonprovisional Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please write to the Application Processing Division's Customer Correction Branch within 10 days of receipt. Please provide a copy of the Filing Receipt with the changes noted thereon.

Applicant(s)

FANG CHEN, NORTH WALES, PA.

CONTINUING DATA AS CLAIMED BY APPLICANT-
PROVISIONAL APPLICATION NO. 60/104,251 10/14/98
PROVISIONAL APPLICATION NO. 60/069,401 12/12/97

IF REQUIRED, FOREIGN FILING LICENSE GRANTED 01/28/99

TITLE
DNA MOLECULES ENCODING HUMAN NUCLEAR RECEPTOR PROTEINS, NNR7 AND NNR7-1

PRELIMINARY CLASS: 435

DATA ENTRY BY: GENTRY, CHRISTINE TEAM: 01 DATE: 03/18/99

(see reverse)

5MH

20084YCA

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
10/090,090	03/04/02	CHEN	20084YCA

MERCK AND CO INC
P O BOX 2000
RAHWAY, NJ ~~07065-0907~~

EXAMINER	
ART UNIT	PAPER NUMBER
	3

DATE MAILED: 1 2 JUN 2002

Reply By 7-27-02

IF NO RESPONSE TO THIS NOTICE IS RECEIVED WITHIN FORTY-FIVE DAYS, A FORMAL REQUIREMENT WILL BE ISSUED

The subject matter of this application appears to:

☒ be "useful in the production or utilization of special nuclear material or atomic energy" as recited in 42 U.S.C. 2182 (Department of Energy (DOE)).

☐ "have significant utility in the conduct of aeronautical and space activities" as recited in 42 U.S.C. 2457 (National Aeronautics and Space Administration (NASA)).

Accordingly, no patent can issue on this application unless applicant(s) file a statement (under oath or in the form of a declaration as provided by 37 CFR 1.68) setting forth (1) the full facts concerning the circumstances under which the invention was made and conceived and (2) the relationship (if any) of the invention to the performance of any work under any contract or other arrangement with the Agency(ies) noted above. On the reverse side of this form is an example of an acceptable format for this statement. The language appearing in paragraphs III and/or IV of the example *must* appear if applicant is attempting to establish that no relationship (under item 2 above) exists.

If the invention disclosed in this application was developed under a contract, grant or cooperative agreement between the Agency indicated above and a person, small business or non-profit organization and rights to the invention have been determined by specific reference to 35 U.S.C. 202 in the contract, grant or cooperative agreement, then applicant need not submit the statement described above. Instead, applicant may file a verified statement (under oath or in the form of a declaration, 37 CFR 1.68) setting forth the information required by 35 U.S.C. 202(c)(6).

IF NO STATEMENT HAS BEEN RECEIVED WITHIN FORTY-FIVE DAYS OF THE MAIL DATE INDICATED ABOVE, a formal requirement for statement will then be issued. No provision is made for extension of the statutory thirty-day period for response to the formal requirement and the penalty for failure to file an acceptable and timely statement is abandonment of the application. Therefore, applicants are strongly encouraged to submit a statement at this time in order to avoid the issuance of a formal requirement.

IT IS IMPORTANT TO NOTE that the statement must accurately represent the property rights situation of the claimed invention if and when the application is found allowable. Thus, if during prosecution before the examiner, the claimed invention is so altered or the property rights situation so changed as to impact the accuracy of a statement submitted earlier, a supplemental statement must be filed. Failure to submit such additional information where appropriate may be considered a false representation of material facts and render the patent owner vulnerable to loss of patent rights and other sanctions as set forth in the statutes. The PTO will not review allowed applications for this possibility. The responsibility for complying with the statutes rests with the applicants.

Any questions regarding this requirement should be directed to Licensing and Review at ~~(703) 308-4191~~. (703) 305-0241

PLEASE DIRECT ALL COMMUNICATIONS RELATING TO THIS MATTER TO THE ATTENTION OF LICENSING AND REVIEW